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TITLE: Novel Combinatorial Immunotherapy for Melanoma

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CONTRACTING ORGANIZATION: Medical college of Wisconsin
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14. ABSTRACT Our research has discovered a novel immune-suppressive ligand protein called <u>V</u> -domain Immunoglobulin Suppressor of <u>T</u> cell <u>A</u> ctivation (VISTA). By directly suppressing T cell-mediated adaptive immune response, VISTA critically impairs anti-tumor immune responses. VISTA monoclonal antibody-mediated blockade synergizes with a peptide vaccine using TLR agonists as adjuvants to potently reject established tumor. Based on these preliminary data, three specific aims are proposed to address the interplay between VISTA pathway and TLR signaling in multiple cell types, namely effector T cells, Foxp ³⁺ CD4 ⁺ regulatory T cells (Tregs), and myeloid cells (i.e. myeloid dendritic cells and myeloid-derived suppressor cells). In this reporting period, we have characterized the role of VISTA in controlling TLR-mediated activation of myeloid cells including DCs and macrophages, and myeloid-derived suppressor cells (Aim 1) and identified potential signaling mechanisms whereby VISTA controls TAK1 activation and regulates the production of inflammatory cytokines. Second, we have established in vitro assay systems to distinguish T cell intrinsic versus extrinsic mechanisms whereby VISTA regulates TCR signaling and T cell activation. In addition, by following adoptively transferred T cells recognizing melanoma antigens, we have confirmed the synergistic activation of T cells in response to combinatorial therapy. These results form the foundation for defining the molecular mechanisms whereby the combinatorial therapy triggers potent anti-tumor T cell responses.					
15. SUBJECT TERMS Cancer vaccine, Immune-checkpoint; VISTA, Toll-like receptor (TLR), vaccine adjuvant, MyD88, Transforming growth factor β -activated kinase 1 (TAK1), Antigen-presenting cells (APC), myeloid-derived suppressor cells, inflammatory cytokines					
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1. INTRODUCTION:

Our research has discovered a novel immune-suppressive ligand protein called V-domain Immunoglobulin Suppressor of T cell Activation (VISTA). By directly suppressing T cell-mediated adaptive immune response, VISTA critically impairs anti-tumor immune responses. Our ongoing research demonstrates the efficacy of VISTA antibody mono-therapy in murine tumor models, against pre-established tumors. Remarkably, VISTA monoclonal antibody-mediated blockade synergizes with a peptide vaccine using TLR agonists as adjuvants to potently reject established tumor, as well as promotes tumor-specific memory T cell response for long-term protection. Based on these preliminary data, we hypothesize that VISTA suppresses the TLR-mediated immune activation via its action on multiple cell types that are directly stimulated by TLR agonists. Three specific aims are proposed to address the interplay between VISTA pathway and TLR signaling in multiple cell types, namely effector T cells, Foxp³⁺CD4⁺ regulatory T cells (Tregs), and myeloid cells (i.e. myeloid dendritic cells and myeloid-derived suppressor cells). By using a clinically relevant inducible melanoma model, we will examine the role of VISTA in establishing the immune-suppressive tumor microenvironment, and how a combinatorial therapeutic platform of VISTA-blockade and TLR-agonists based vaccine is effective for treating established melanoma.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Cancer vaccine, Immune-checkpoint; VISTA, Toll-like receptor agonist, vaccine adjuvant, MyD88, dendritic cells, macrophages, myeloid-derived suppressor cells (MDSC), inflammatory cytokines

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim-1 (1-12 months) major goals

➤ **Task-1: optimize the use of TLR agonists**

Target dates: 1-3 months; Status: completed.

Accomplishment:

Because of the toxicity of TLR agonists as vaccine adjuvant, one of the goals in this proposal is to optimize the use of TLR agonists and define the minimal components that effectively synergize with VISTA-blocking mAb and peptide vaccine. To this end, we have compared the therapy using VISTA-blocking mAb together with a peptide vaccine and either 4 TLR agonists cocktail containing CpG (specific for TLR9), R848 (specific for TLR7/8), LPS (for TLR4) and poly (I:C) (for TLR3), or a 2-TLR agonists cocktail containing CpG and R848.

Our data show that the CpG/R848 cocktail effectively synergized with the rest of the therapeutic components and achieved similar efficacy when compared to the 4 TLR cocktail in the B16 melanoma model. (**Fig. 1**)

Future plan:

For the rest of the studies, we will focus on defining the mechanisms of synergy between VISTA blockade and 2 TLR agonists: CpG and R848.

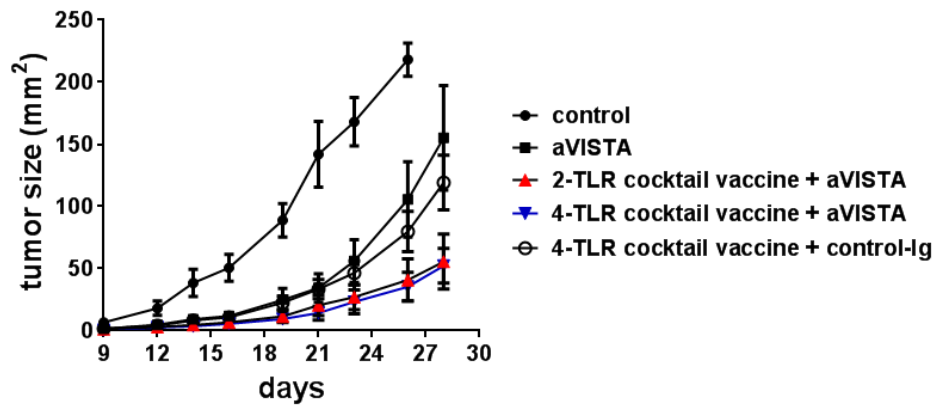


Figure 1. TLR agonists CpG and R848 effectively synergized with a peptide vaccine and VISTA-blocking mAb in melanoma model. Mice were inoculated with B16 melanoma (50k) and were treated on day+3 as indicated, with either VISTA-blocking mAb, or TRP1/TRP2-derived peptide vaccine containing 4-TLR agonist cocktail of CpG (50 mg), R848 (50 mg), poly (I:C) (100 mg), and LPS (25 mg), or 2-TLR agonist cocktail of CpG and R848. When indicated, combined treatment was performed.

➤ **Task-2: Examine the suppressive impact of VISTA-ECD on DC activation in response to TLR agonists**

Target dates: 1-6 months; Status: completed

Accomplishment:

We isolated DCs from WT and VISTA^{KO} mice and stimulated cells with TLR9 agonist CpG. Culture supernatants were harvested after 4 hrs and the levels of cytokines (IL6 and IL12) were examined by ELISA. Our data show that immobilized VISTA-ECD is capable of suppressing the production of cytokines such as IL6, MCP-1, and IL1b from WT or VISTA^{KO} DCs. These data indicate that VISTA acts as a ligand that engages an inhibitory receptor that suppresses TLR signaling (**Fig. 2**).

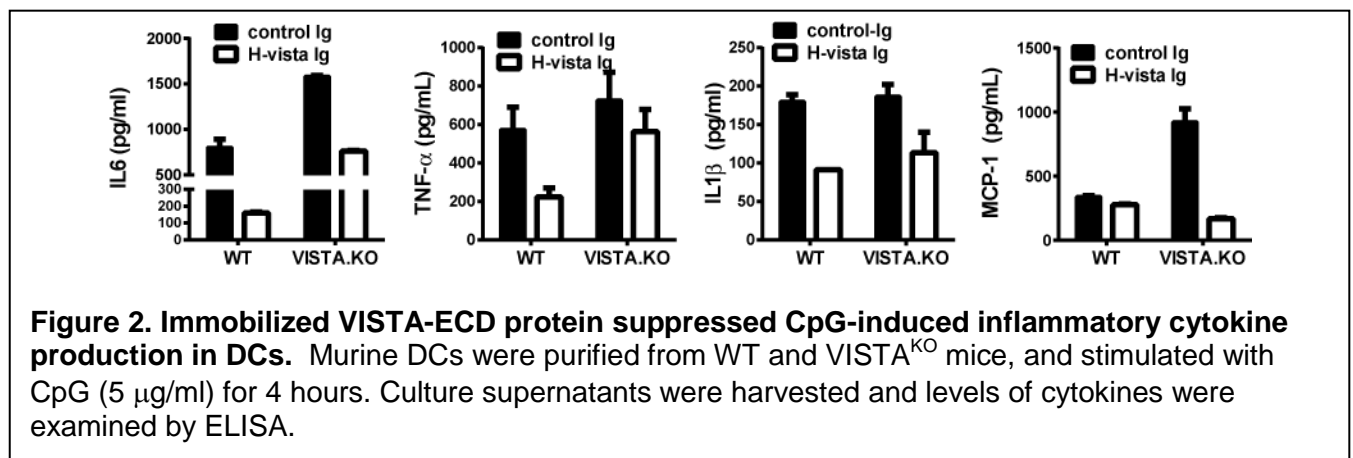


Figure 2. Immobilized VISTA-ECD protein suppressed CpG-induced inflammatory cytokine production in DCs. Murine DCs were purified from WT and VISTA^{KO} mice, and stimulated with CpG (5 µg/ml) for 4 hours. Culture supernatants were harvested and levels of cytokines were examined by ELISA.

➤ **Task-3: Examine the synergistic activation of DCs in response to TLR agonists and VISTA blockade**

Target dates: 3-9 months; 60% completion

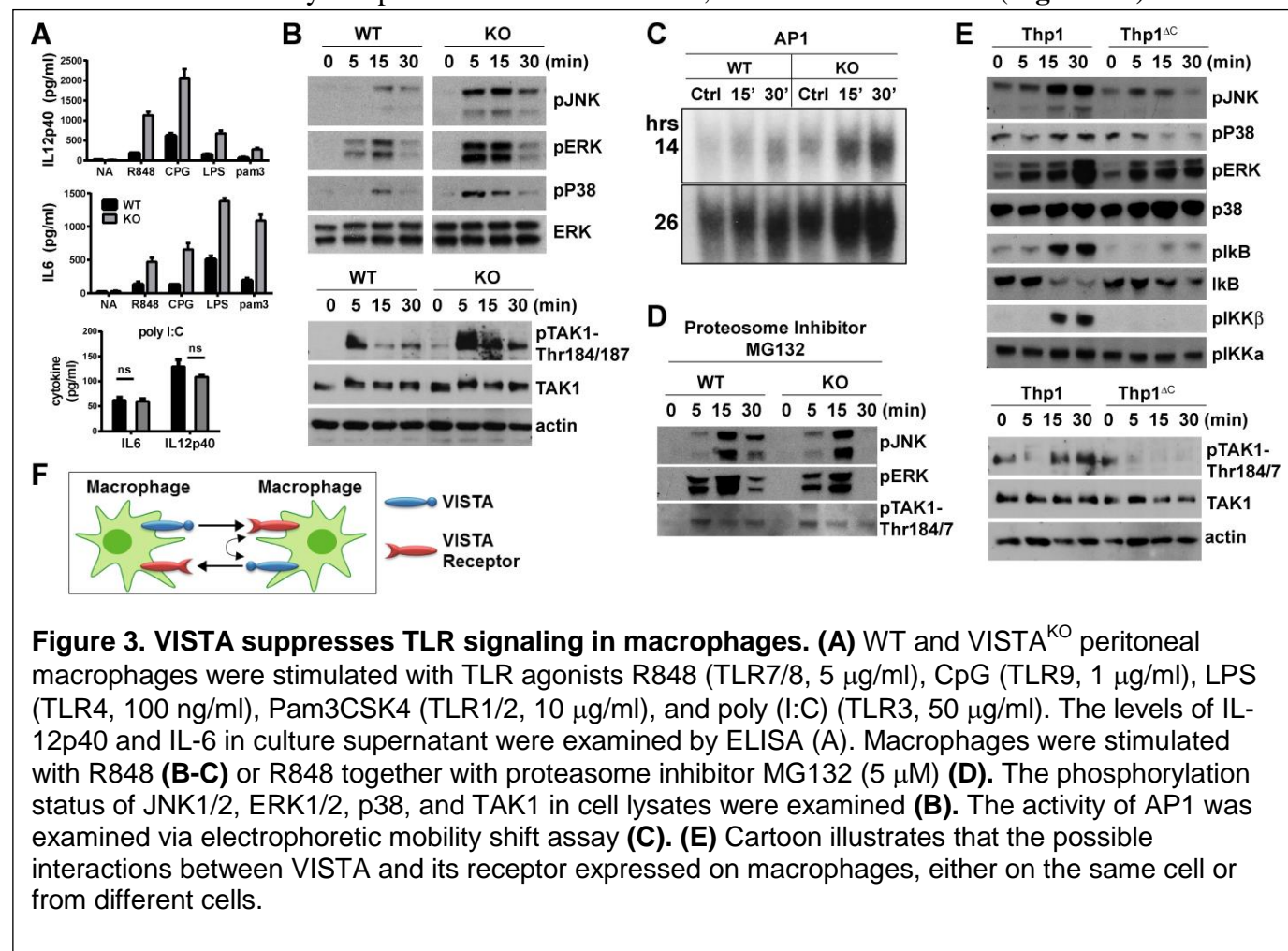
Accomplishment:

Murine DCs are composed of a mixed population of cd11b-hi, CD11b-int, and CD11b-negative cells, which contain heterogeneous levels of VISTA expression that correlates with the level of CD11b expression. This heterogeneity prevents us to accurately analyze the impact of VISTA on the function of DCs.

In order to circumvent this issue and gain insights to the mechanisms regarding how VISTA regulates TLR signaling and controls the efficacy of TLR agonists as vaccine adjuvant, we took advantage of murine macrophages, which express high levels of VISTA.

We examined TLR-signaling in peritoneal macrophages isolated from WT and VISTA^{KO} mice. Our data show that the engagement of VISTA to its receptor, expressed on macrophages, suppressed the production of inflammatory cytokines downstream of TLR signaling, via reducing the phosphorylation of the transforming growth factor β -activated kinase 1 (TAK1) (**Fig. 3**).

The outcome of TAK1-mediated signaling regulates cytokine production from myeloid cells, T and NK lymphocytes, therefore controlling the inflammatory status of the tumor microenvironment (TME). Our data shows that VISTA^{KO} macrophages produced higher levels of cytokines in response to TLR agonists R848, CpG, LPS and Pam3, but not poly(I:C), indicating that VISTA inhibits MyD88-dependent signaling (**Fig. 3A**). Consistently, enhanced phosphorylation of TAK1-Thr184/187 and JNK/ERK/p38 MAPKs, and potent AP1 activation was observed in VISTA^{KO} macrophages (**Fig. 3B-C**). Pre-treatment with the proteasome inhibitor MG132 abolished the hyper-responsiveness in VISTA^{KO} macrophages (**Fig. 3D**), indicating a potential role of ubiquitination and turnover of signaling proteins in mediating the suppressive function of VISTA receptor. Together, these data support the hypothesis that VISTA acts as a ligand that engages a yet-to-be-identified inhibitory receptor either on the same cell, or from a different cell (**Figure 3E**).



Future plan:

For the next reporting period, we plan to:

a) Confirm the regulatory role of VISTA in controlling TLR signaling in myeloid DCs, which play a critical role in mediating the efficacy of our combinatorial therapeutic regime. Given the heterogeneity of VISTA expression level on DC populations, we will design appropriate assays to distinguish the response from VISTA-negative or low DCs from VISTA-hi DCs.

b) We will continue to analyze the signaling events downstream of TLR and determine the molecular targets of VISTA and its receptor. We will dissect how VISTA regulates the MyD88-IRAK-TRAF6-TAK1 signaling axis, and the assembly and function of the CARD9-Bcl10-Malt1 (CBM) signalosome.

c) Since our data show that the activity of proteasome is required for the augmented TLR signaling in VISTA^{KO} cells, we hypothesize that VISTA receptor controls TLR signaling via altering the ubiquitination and turnover of key signaling proteins. We will test the role of ubiquitin-modifying enzymes and phosphatases in mediating the inhibitory signaling of VISTA receptor.

➤ **Task-4: Examine the phenotype and the suppressive function of MDSCs in response to combination therapy**

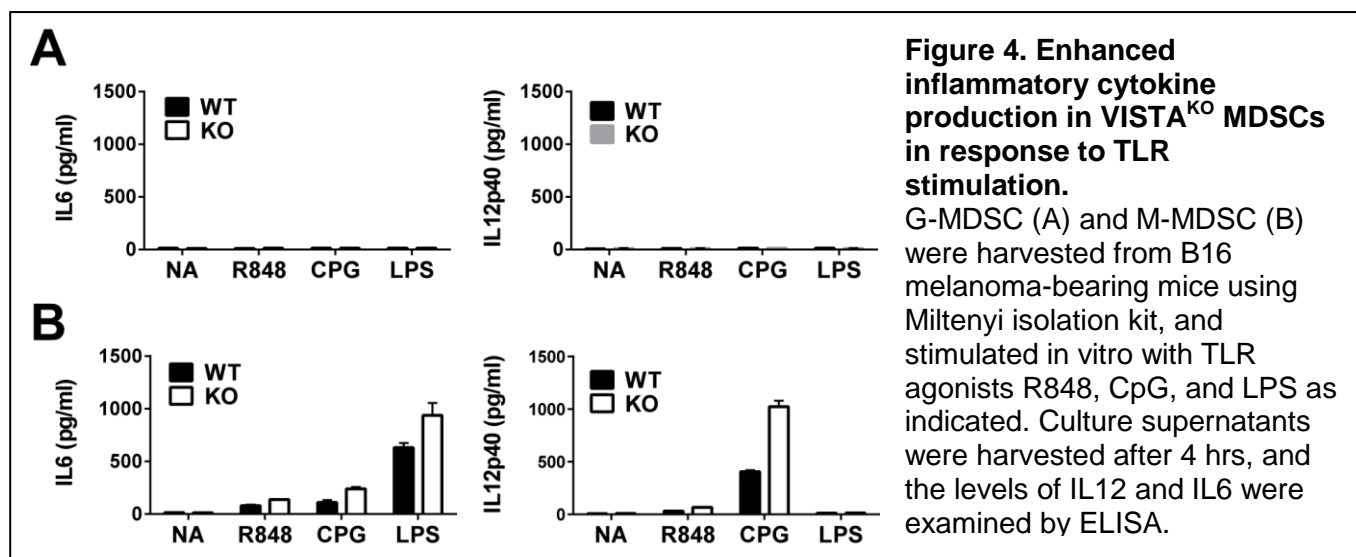
Target dates: 3-12 months; 60% completion

Accomplishment:

To complete this task, we first isolated granulolytic (Cd11b⁺ Ly6G⁺ G-MDSC) and monocytic MDSCs (Cd11b⁺ Ly6C⁺ M-MDSC) from WT and VISTA^{KO} melanoma-bearing mice, and stimulated cells with TLR agonists CpG, R848 and LPS. Culture supernatants were harvested after 4 hrs and the amount of secreted cytokines were examined by ELISA. Our data show that only M-MDSCs produced significant levels of inflammatory cytokines such as IL12 and IL6. VISTA^{KO} M-MDSC cells produced higher levels of cytokines than WT M-MDSCs, indicating that the lack of VISTA could potentially convert M-MDSCs into pro-inflammatory and T-stimulatory phenotypes, thus contributing to the tumor-clearing efficacy of the combinatorial therapy (**Fig. 4**)

Future plan:

We are in the process to set up the MDSC-T cell co-culture assay to quantify the suppressive function of WT and VISTA^{KO} MDSCs before and after treatment with TLR agonists.



➤ **Task-5: Examine the synergistic activation of human monocytes**

Target date: 6-12 months; 60% completion.

Accomplishment:

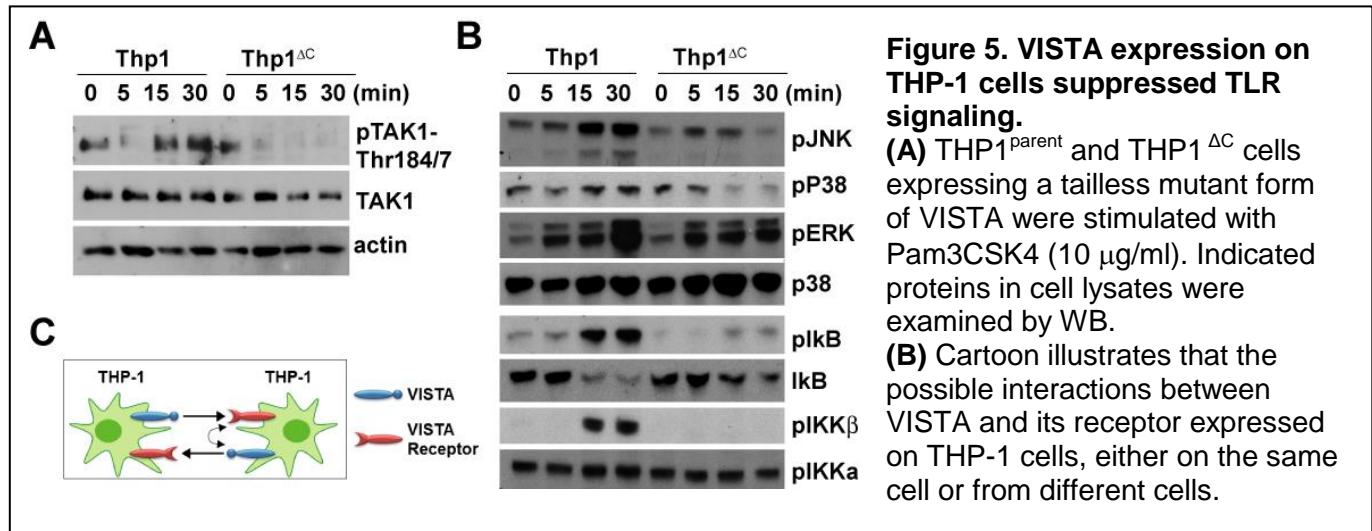
Due to the complex procedure of obtaining a steady source of human PBMCs, we have performed pilot experiments using a human monocyte cell line THP1 to examine the regulatory role of VISTA on TLR signaling in human monocytes. Our result show that overexpression of a tailless mutant form of VISTA potently suppressed the activation of THP1 cells, which lacks endogenous VISTA, in response to TLR3

agonist Pam3csk4 (**Fig. 5**). VISTA potently ablated the phosphorylation of TAK1, as well as the phosphorylation of JNK/ERK/p38 MAPKs, and those of IKK α and I κ B (**Fig. 5A-B**).

Taken together, these data indicate that VISTA acts as a ligand that engages an inhibitory receptor, which in turn controls the activation of TAK1 and inflammatory cytokine production in response to TLR stimulation.

Future plan:

- 1) We will continue to define the molecular mechanisms whereby VISTA and its receptor controls MyD88-mediated TAK1 activation and TLR signaling, using THP1 cells as a model system.
- 2) We will validate our results using primary human monocytes isolated from PBMCs.



Specific Aim 2 (3-18 months)

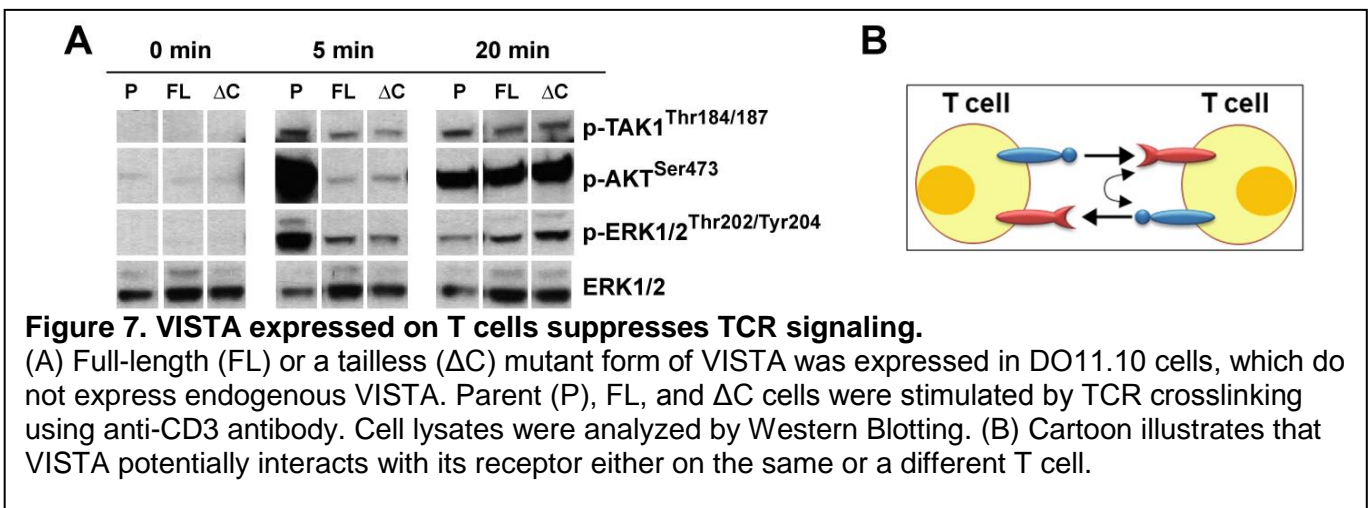
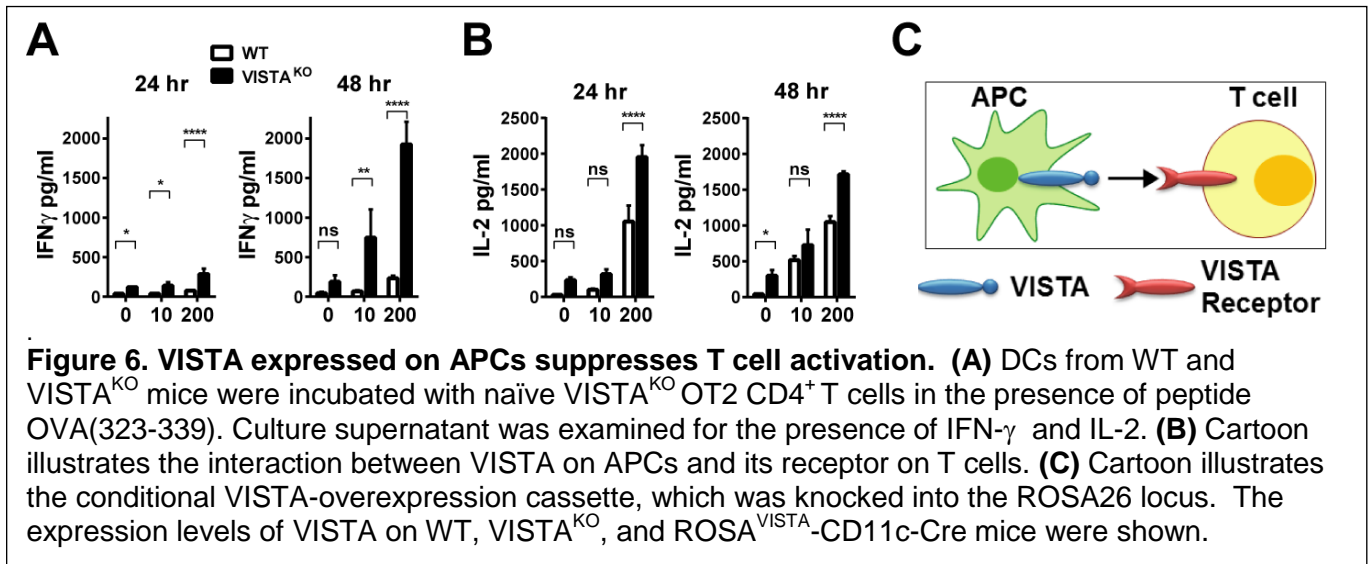
- **Task 1 :** Distinguish T-cell intrinsic versus extrinsic mechanisms of synergy:
 Target dates: 3-12 months; Status: 80% completion.

Accomplishment:

We have designed distinct assay systems to distinguish the role of VISTA on APCs (T-cell extrinsic) versus its role on T cells (T-cell intrinsic).

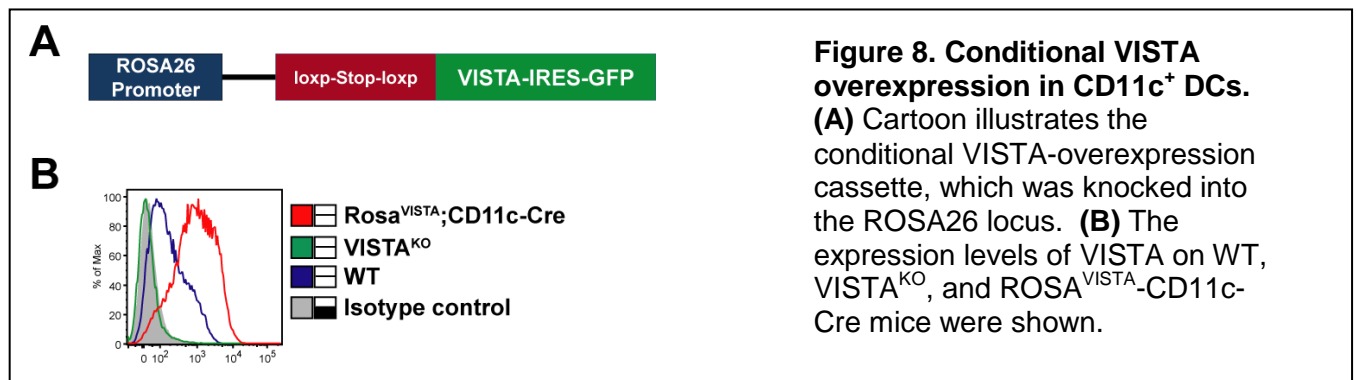
Our earlier studies show that immobilized soluble VISTA-Ig protein containing the extracellular domain (ECD) suppressed the phosphorylation of multiple proximal TCR signaling proteins. Since plate-bound suppression assay does not fully recapitulate the APC:T cell interactions, we employed an ex vivo co-culture assay to define the mechanisms of action (**Fig. 6**). We isolated WT and VISTA^{KO} DCs, and stimulated VISTA^{KO} T cells. Our data show that VISTA expression on DCs inhibited the production of IFN- γ and IL-2 from T cells (**Fig. 6A and B**). The cartoon illustrates the interaction between VISTA expressed on DCs and a transmembrane receptor on T cells (**Fig. 6C**).

In addition to being expressed as a ligand on APC, VISTA is constitutively expressed on naïve T cells. It contains a 98 aa cytoplasmic domain, which may transmit inhibitory signals. This notion was recently proposed by Dr. Lieping Chen's group (Flies et al. (2014) Coinhibitory receptor PD-1H preferentially suppresses CD4⁺ T cell-mediated immunity. *J Clin Invest*). However, using DO11.10 T cells expressing a tailless mutant VISTA, our data show that VISTA-ECD, but not the cytoplasmic domain of VISTA is sufficient to suppress T cell-activation, indicating that VISTA-ECD interacts with VISTA receptor either on the same T cell, or from a different T cell (**Fig. 7**). This assay system therefore allows us to determine the regulatory role of VISTA on T cell activation in a T cell-autonomous manner.



Future plan:

First, since the level of VISTA expression on DCs is heterogeneous, which prevents an accurate evaluation of its function, we generated a transgenic mouse strain that carries a Flox-STOP-Flox-VISTA-IRES-GFP expression cassette knocked into the ROSA26 promoter (**Fig. 8A**). Upon breeding with the CD11c-Cre transgenic mice, the $ROSA^{VISTA}$ -CD11c-Cre mice showed uniform GFP expression in DCs, as well as higher levels of VISTA expression than WT DCs (**Fig. 8B**). We will therefore use DCs isolated from WT, $VISTA^{KO}$, and $ROSA^{VISTA}$ -CD11c-Cre mice to define the molecular mechanisms whereby VISTA acts as an inhibitory ligand on APCs to suppress TCR signaling in the presence and absence of TLR stimulation.



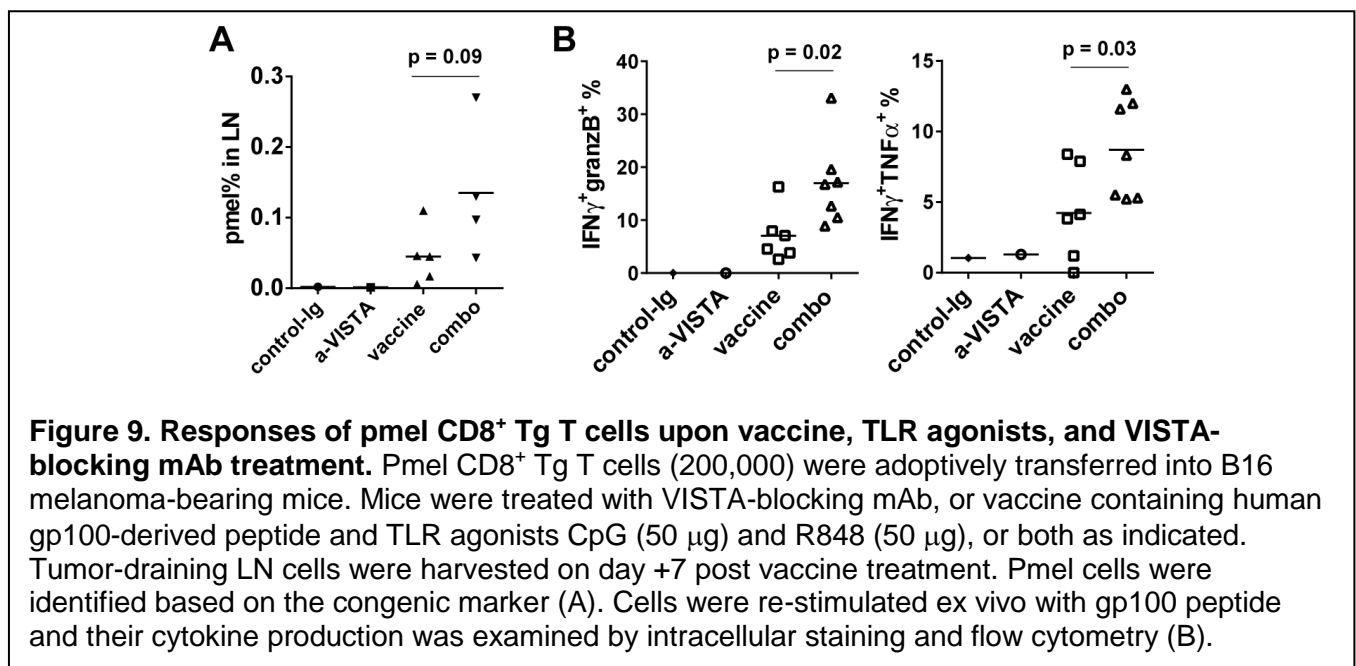
2nd, we will use the DO11.10 assay system to examine T-cell intrinsic role of VISTA on TCR signaling, in the presence and absence of TLR stimulation.

- **Task 2:** Modeling T-cell responses using melanoma antigen specific transgenic T cells:
Target dates: 3-12 months; Status: 50% completion.

Accomplishment:

To model anti-tumor T cell responses using melanoma antigen specific transgenic T cells, we have adoptively transferred pmel CD8⁺ transgenic (Tg) T cells (specific for melanoma antigen gp100) into tumor-bearing host. Upon treatment with the gp100-derived peptide vaccine together with TLR agonist CpG and R848, we examined the response of Pmel cells in the presence or absence of VISTA. Our data show augmented accumulation (**Fig. 9A**) and increased production of effector cytokines (IFN γ , TNF α , and granzyme B) of Pmel cells in tumor draining LN upon anti-VISTA mAb treatment (**Fig. 9B**). These data are consistent with the hypothesis that VISTA

Future plan: We have acquired high-affinity and low-affinity TRP2 Tg CD8⁺ T cells from our collaborator Dr. Arthur Hurwitz (NCI). We are currently breeding these mice and will perform similar analysis as shown for Pmel CD8⁺ cells, to determine whether VISTA mAb treatment can enhance the responses of both high and low affinity T cells.



- **Task 3:** Examine the synergistic activation of human T cells
Target dates: 12-18 months; Status: 0% completion.
Future plan: This task will be carried out for the next reporting period.

Specific Aim 3 (12-24 months)

- **Major Task 1 :** Examine the homeostasis and phenotypic alterations of Tregs in response to combination therapy
- **Major Task 2:** Examine whether combination therapy maximally abolishes the suppressive function of Tregs
- **Major Task 3:** Examine the de novo induction and suppressive function of adaptive Tregs

Target dates: 12-24 months; Status: 0% completion.

Future plan: The tasks in Aim 3 will be carried out for the next reporting period.

What opportunities for training and professional development has the project provided?

We have provided the following training opportunities for Dr. Wenna Chen and Dr. Na Li:

1. Since they are international scholars, to improve their language proficiency, both of them were enrolled in the English language class provided by Medical College of Wisconsin and Blood Research Institute (BRI). The class provides training in English speaking, writing, and power point presentations.
2. To improve public speaking and presentation skills, both of them have participated in the weekly research-in-progress seminar series, hosted by the department, and have given presentations regarding their research projects.
3. To improve networking with the research community at MCW and BRI, as well as improve communication skills, Na has participated in the annual “Graduate student and Postdoctoral Fellow poster day” event (Oct 22nd, 2015), as well as the annual “Immunology Community Retreat” event organized by BRI (Oct 14th, 2015), and successfully presented her research data.
4. The department of Microbiology & Molecular Genetics hosts weekly seminar given by invited external speakers, who present cutting edge research related to Tumor Immunology and Cancer Immunotherapy. Na has participated in meetings with several seminar speakers, introducing to them her research projects and . and participated in round-table discussions regarding career development.
5. The department of Microbiology & Molecular Genetics hosts once a month seminar series regarding career development for graduate students and postdoctoral fellows. Topics range from scientific writing for manuscripts and grant applications, to research integrity and ethics, to alternative career development. Both Wenna and Na have participated in those seminars.
6. As part of the daily training process, our laboratory has maintained weekly group meetings, during which an hour long power point presentation of the up-to-date data is presented. One-on-one discussions occur not only during the group meeting, but also during daily experimental planning and discussion of data.

How were the results disseminated to communities of interest?

Nothing to report.

4. IMPACT: This component is used to describe ways in which the work, findings, and specific products of the project have had an impact during this reporting period. Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

Immune-checkpoint proteins such as CTLA-4 and PD-1 impair anti-tumor immune responses. Antibodies inhibiting these molecules have become breakthrough therapies for cancer. However, the overall response rate is still as low as ~20-30% among cancer patients. Novel therapeutic targets and approaches are urgently needed

to improve the efficacy of cancer immunotherapy. VISTA is a novel immune-checkpoint and a promising new therapeutic target. VISTA-blocking antibody is an innovative future clinical drug for cancer treatment.

Our studies in this reporting period have the following impact:

1. Using preclinical tumor models, we have tested and identified the minimum effective combinations of TLR agonists that effectively synergize with VISTA-blocking mAb. This result will guide and facilitate the future clinical translation of applying this regime for cancer treatment.
2. Using in vitro culture assays, we have validated that VISTA directly suppresses TLR signaling, via regulating the phosphorylation of key signaling component TAK1. This novel finding will establish VISTA not only as a regulator of T-cell mediated adaptive immune response, but also a critical regulator of TLR-mediated innate immune response. This finding directly impacts on the cancer vaccine design when incorporating TLR agonists as adjuvants.
3. Analysis of MDSC indicates that VISTA-blockade effectively enhanced/alters the response of these immune suppressive cells to become pro-inflammatory. This novel regulatory role of VISTA on MDSC brings a better understanding regarding the mechanisms of our proposed combinatorial therapy.
4. VISTA receptor is a yet-to-be-identified transmembrane receptor. Our newly established THP-1 cell line model uses TLR signaling as a functional readout, to directly measure the suppressive function of VISTA-mediated signaling. This assay provides us with a feasible platform for the design of a high-throughput screening strategy for the identification of the VISTA receptor in future studies.
5. Similarly, the THP-1 system allows us to identify molecular targets downstream of VISTA receptor, in the context of TLR signaling. For the next reporting period, it will be possible for us to use approaches including candidate-based validation (i.e. ubiquitin-modifying enzymes and phosphatases), as well as unbiased screening of sh-RNA or CRISPR libraries to identify novel regulatory molecules downstream of VISTA. These studies eventually will lead to the identification of novel therapeutic targets for cancer immunotherapy.
6. Using preclinical models, we have defined and correlated the functional parameters of tumor-specific T cells to the tumor-clearing efficacy of the therapy. These parameters will guide the immune monitoring for future clinical trials.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Currently, the intellectual property related to VISTA-specific blocking agents (such as monoclonal antibody, mAb) has been licensed to the Janssen Biotechnology Inc, who will develop fully humanized anti-VISTA mAb for cancer immunotherapy. The results from this study will form the foundation for filing a patent application regarding the combinatorial therapy combining VISTA-blocking mAb, cancer vaccine and TLR agonists as adjuvants. These results will eventually lead to the clinical application of this combination therapy for cancer treatment.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

- Changes in approach and reasons for change: nothing to report
- Actual or anticipated problems or delays and actions or plans to resolve them:

Due to family obligations, Dr. Wenna Chen left our laboratory in October of 2014 to join her family in Canada. As a result, the proposed studies in this project were delayed until March 2015, when Dr. Na Li and Dr. Wenwen Xu joined the lab. We have since then made great progress on this project and expect to complete the stated SOW. We have formally submitted a personnel change request to allow Dr. Na Li replace Dr. Wenna Chen's position.

- Changes that have a significant impact on expenditures: nothing to report
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. Examples of products include:

- **Publications, conference papers, and presentations**

Poster presentations at the Center for Human Immunology Symposium and Annual Immunology Retreat, MCW, 2015

1. Immune-checkpoint protein VISTA negatively regulates Toll-like receptor-mediated signaling and impairs the therapeutic efficacy of cancer vaccine using TLR agonists as adjuvant

Authors: Wenwen Xu¹, Na Li¹, Ying Yuan^{1,2}, Yongwei Zheng^{1,5}, Peter Volberding¹, Kamal Rajasekaran^{1,3,4,5}, Halli Miller¹, Michael Olson¹, Austin Schenk⁶, Deming Wang^{1,5}, Subramaniam Malarkannan^{1,3,4,5}, Li Wang¹

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Li Wang
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	eRA Commons User ID LIWANG01
Nearest person month worked:	4 academic months
Contribution to Project:	Dr. Wang has supervised the project, worked with postdoctoral fellows Wenwen and Na to carry out proposed studies.
Funding Support:	R01CA164225 and W81XWH-14-1-0587

Name:	Marc Ernstoff
Project Role:	designated collaborator
Researcher Identifier (e.g. ORCID ID):	eRA Commons User ID
Nearest person month worked:	0.6 academic months
Contribution to Project:	Dr. Ernstoff has served as a designated collaborator, and facilitated the proposed studies by providing consultations and insightful discussions regarding study designs and data analysis.
Funding Support:	Cancer Immunotherapy Trial Network (NCI)

Name:	Na Li
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	eRA Commons User ID Nali0612
Nearest person month worked:	8 academic months
Contribution to Project:	Dr. Na Li has worked with Dr. Wang (PI) and Wenwen Xu (postdoc) to carry out proposed studies as described in SOW.

Funding Support:

W81XWH-14-1-0587

Name: Wenwen Xu
Project Role: Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID): eRA Commons User ID wenwenxu
Nearest person month worked: 4 academic months
Contribution to Project: Dr. Wenwen Xu has worked with Dr. Wang (PI) and Dr. Na Li to carry out proposed studies
Funding Support: R01CA164225

Name: Wenna Chen
Project Role: Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID): unknown
Nearest person month worked: 1 academic month
Contribution to Project: Dr. Wenwen Xu has worked with Dr. Wang (PI) on proposed studies.
Funding Support: W81XWH-14-1-0587

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.